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Influence of Decreased Solvent Permittivity on the Structure and Magnesium(II)-Binding Properties of the Catalytic Domain 5 of a Group II Intron Ribozyme

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received …

This study is dedicated to Bernhard Lippert (University of Dortmund), an outstanding advisor and friend (R.K.O.S.) with admiration for his seminal work on the chemistry of nucleobases and with the best wishes for all his future endeavours from all the authors.

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Abstract

Although it is well known that the so-called "equivalent solution" or "effective" solvent permittivity (dielectric constant) in proteins and nucleic acids is lower than in bulk water, this fact is commonly neglected in (bioinorganic) studies of such compounds. Using domain 5 of the group II intron ribozyme Sc.ai5γ, we describe here the influence of 1,4-dioxane-*d8* on the structure and magnesium(II)-binding properties of this catalytic domain. Applying one- and two-dimensional NMR, we observe distinct structural changes in the functionally important bulge region following a decrease in solvent permittivity. Concomitantly, an increase by a factor of 1.5 in the affinity of Mg^{2+} towards the individual binding sites in the catalytic core domain is observed upon addition of 1,4-dioxane-*d8*. This has led to the detection of a new metal ion coordination site near the GU wobble pair in the catalytic triad. Our results show that solvent permittivity is an important factor in the formation of intrinsic RNA structures and affects their metal ion binding properties. Hence, solvent permittivity should be taken into account in future studies.

Keywords: RNA, NMR, solvent permittivity, dielectric constant, metal ion binding

1. Introduction

Group II introns are large metallo-ribozymes with a size ranging between 600 and 2500 nucleotides that were discovered in protists, fungi, algae, and plants as well as in some prokaryotic organisms [1,2]. These large molecular machines are self-splicing introns catalyzing their own removal from the pre-RNA initially formed during transcription. In higher eukaryotes, this process is usually performed by the spliceosome, a huge RNA-protein complex consisting of five small RNAs and dozens of proteins [3]. In contrast to the spliceosome, group II introns show a larger variety of reactions, e.g. these ribozymes are capable of reinserting themselves into RNA or DNA, thus rendering them mobile genetic elements [1,2,4]. All group II introns possess a conserved secondary structure consisting of six domains, which all have specific functions for folding and catalysis. Catalytically important nucleotides are thereby spread out over the whole sequence totalling in minimum about 600 building blocks. Unlike the other domains, domain 5 (D5) is highly conserved in sequence and length. It forms a very stable hairpin of mostly 34 nucleotides and represents the most essential active-site part of all group II introns [1,2]. It is remarkable that the individual domains of group II introns are independent folding units that reassemble to the full active structure when added *in trans* to each other [2,5]. It has been shown that metal ions thereby occupy the same sites in the free domains as well as in the assembled three dimensional architecture of the fully functional ribozyme [6].

Metal ions play a crucial role in folding und function of any RNA and consequently also in those of group II introns [7]. Due to the polyanionic phosphate-sugar backbone of nucleic acids charge-screening is necessary to allow the formation of secondary structure elements. This task is most probably performed predominantly by monovalent ions. In addition, monovalent ions, especially K^+, are potentially included in the stabilization of tertiary RNA structures as well [8,9]. Nevertheless, divalent metal ions, in particular Mg^{2+}, are normally responsible for the formation of tertiary structural elements. It is well known that, besides the presence of RNA-binding proteins and polyamines, the kind and concentration of metal ions have a significant impact on the three-

dimensional structure of a given RNA [7,10-12]. In the case of ribozymes divalent metal ions are in addition implicated in catalysis [7,13-15] and in most ribozyme structures they are found close to the catalytic core. It is relevant to mention in this context that labile and biologically relevant metal ions can coordinate to RNA in close neighbourhood to each other [16,17] and even to the same nucleobase [18]. Metal ions thereby fulfil different functions in the various ribozymes [7,11,14]: They can activate water or 2'-OH groups as nucleophiles for phosphodiester cleavage, orient ribose and phosphate properly for the cleavage and stabilize the transition state or the cleavage products, respectively [10,14,19]. In addition, divalent metal ions can cause significant pK_a shifts at amino and imino protons [20] and thus strengthen hydrogen bonds [21-27] or stabilize unusual base pairs [23] or rare tautomers [28].

There are numerous studies that deal with the metal ion-binding properties of group II introns [7,10,14,29-35]. These studies were usually performed in aqueous buffer solutions exhibiting a dielectric constant (permittivity) close to the one of water ($\varepsilon \approx 78.5$; 25 °C). However, it is well known that the so-called "equivalent solution" or "effective" dielectric constant in proteins [36-43] and particularly in the active-site cavities of metalloenzymes [44] is significantly lower and varies roughly between 30 and 70 [36,38,44]. Comparably, estimates for the dielectric constant at the surface of nucleic acids range from about 20 to 66 [45-51]. In this context it is noteworthy that the permittivity varies clearly within a single nucleic acid. Theoretical investigations into the local dielectric environment of B-DNA showed a decrease in the dielectric constant from the phosphate backbone ($\varepsilon = 66$) to the major ($\varepsilon = 53$) and minor ($\varepsilon = 51$) groove [49].

Bearing the above observations in mind and based on recent studies [31], we here investigate by NMR spectroscopy the influence of a solvent being less polar than water on the structure and Mg^{2+}-binding properties of RNA, using domain 5 of the group II intron ribozyme Sc.ai5γ located in the *cox1* gene of the yeast *Saccharomyces cerevisiae* (Fig. 1).

<<Fig. 1 close to here>>

Changes in solvent permittivity are of special relevance for the proton- and metal ion-

binding properties of nucleic acids [11]. In the case of oxygen ligands both, the basicity of the ligand as well as the stability of its complexes, increase drastically as the solvent polarity decreases [52], while for nitrogen ligands a decreasing solvent polarity causes a considerable decrease of basicity, but has just a moderate effect on complex stability [53,54]. Due to the abundance of phosphate and carbonyl oxygen atoms as ligands one can thus expect an increase in the stability of metal ion complexes of nucleic acids and their constituents upon reduction of the solvent permittivity as has been repeatedly [55-59] verified experimentally with their building blocks. To tune the dielectric constant of the solvent we used mixtures of 1,4-dioxane-*d8* ($\varepsilon \approx 2$; 25 °C) and water ($\varepsilon \approx 78.5$; 25 °C) [60,61]. 1,4-dioxane-*d8* has been chosen due to its low dielectric constant, its total miscibility with water and its comparably weak (hydrophobic) solvating properties [59]. On the other hand the solubility of RNA in dioxane-water mixtures is significantly reduced in comparison to the one in water, thus only mixtures containing up to 20% (*v/v*) of the organic solvent could be used in our NMR studies. Nevertheless, the permittivity of these solvent mixtures ($\varepsilon = 61 – 78$) allows us to mimic to a certain extent the natural environment of this ribozyme domain, as D5 is located within the hydrophobic core of the three-dimensional architecture of the catalytically active RNA structure [62] being surrounded by the other five domains.

2. Experimental

The 5'-triphosphates of adenosine (ATP), cytidine (CTP) and guanosine (GTP) were from GE Healthcare (Chalfont St. Giles, UK), uridine 5'-triphosphate was obtained from Acros (Geel, Belgium). In addition, CTP and UTP, as well as magnesium chloride (99.99%), were purchased from Sigma-Aldrich (St. Louis, USA). DNA oligonucleotides were from Operon (Cologne, Germany) or Microsynth (Balgach, Switzerland). Aqueous acrylamide solution (AccuGel 29:1) and TBE buffer (89 mM Tris, 89 mM H_3BO_3, 2 mM EDTA; pH 8.3) used for RNA and DNA purification were obtained from National Diagnostics (Hussle Hull, UK), urea (UltraPure) from Eurobio (Les Ulis, France). The deuterated reagents 1,4-dioxane-*d8* (99% D), NaOD (40% in D_2O;

99.9% D) and DCl (38% in D_2O; 99.5% D) were purchased from Armar Chemicals (Doettlingen, Switzerland), whereas D_2O (99.998% D) was from Cambridge Isotope Laboratories (Andover, USA). All other chemicals were obtained from Sigma-Aldrich and were used without further purification. DNA oligonucleotides were purified by PAGE, followed by electroelution with a *Biotrap* device using BT1 and BT2 Elutrap membranes (all from Whatman, London, UK), and ethanol precipitation [63]. For desalting Vivaspin 2 concentrators with a molecular weight cut-off of 3 kD (VivaScience, Hannover, Germany) were used. The concentration of RNA, DNA and NTP solutions were determined by measuring the UV absorption at 260 nm. The extinction coefficients ε_{260} of the nucleic acid strands were calculated using the nearest-neighbour method [64].

D5 was prepared as described recently [31,63] by *in vitro* transcription from double stranded DNA using home-made T7 polymerase [63,65,66]. The RNA was purified by denaturing (7 M urea) PAGE, recovered by electroelution, desalted, and lyophilized. For NMR measurements in D_2O/dioxane-*d8* mixtures the samples were dissolved in D_2O and re-lyophilized in order to minimise the HOD content. For NMR spectroscopy the RNA was dissolved either in H_2O/D_2O (9:1) or D_2O and containing a final concentration of 100 mM KCl (I = 0.1 M) and 10 μM EDTA, respectively. The solutions were brought to pH 6.4 for samples in H_2O/D_2O and to pD 6.8 for samples in D_2O (see above), using DCl or NaOD stock solutions. Dioxane-*d8* (0 – 20% *v/v*) was added last to avoid evaporation of the volatile solvent. The total volume of the sample was consistently 220 μL, the RNA concentrations varied between 0.6 and 1.0 mM.

NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer using a 5 mm BBI probehead or a Bruker AV 600 MHz spectrometer equipped with a CP-TCI triple resonance cryoprobe. [^{1}H,^{1}H]-NOESY spectra were recorded at 303 K in D_2O using a mixing time of 250 ms to assign non-exchangeable proton resonances. Titrations with Mg^{2+} were performed by monitoring exchangeable proton resonances in 9:1 H_2O/D_2O and 9:1 H_2O/1,4-dioxane-*d8* by [^{1}H]-NMR spectra at 278 K using a watergate H_2O suppression. The chemical shift changes of the protons were observed in the presence of 0, 0.5, 1, 2, 4, 6 and 8 mM $MgCl_2$. All spectra were processed with

XWINNMR or TopSpin (Version 1.2 or 1.3) and evaluated using Sparky [67] for two-dimensional and MestReC (Mestrelab Research, Santiago de Compostela, Spain) for one-dimensional spectra.

3. Results and Discussion

3.1. A decreased solvent permittivity changes the structure of D5

We used in our studies the same construct (Fig. 1a) of which the solution structure of D5 was recently solved [31]. The terminal G-C base pair (G0 and G35) that was introduced in order to improve the yield from *in vitro* transcription is known not to influence the structure of the domain [31]. D5 forms a stable hairpin structure that is closed at one end by a GAAA tetraloop and consists of two helical regions that are separated by a bulge of four unpaired nucleosides (U9, A24, C25 and G26). Helix 1 (G1 to A8 and U27 to C34) includes a highly conserved AGC triad (A2, G3 and C4) that is crucial for the catalytic activity of the ribozyme and is thus often designated as catalytic triad. While the structure of the tetraloop does not differ significantly from those of other GNRA loops, the bulge, being rather flexible and largely exposed to solvent, exhibits some interesting structural features [31]. G26 adopts an unusual *syn* conformation and flips down into the major groove of helix 1, where it approaches the catalytic triad. This leads to a kink in the backbone near G26, exposing the base plane of the adjacent A-U pair (A8 and U27) to the solvent. The bulge nucleobases A24 and C25 thereby stack onto helix 2 as shown by a number of characteristic NOE crosspeaks including a distinct interstrand NOE between G10H1' and A24H2 (Fig. 1b) [31]. Overall, this RNA hairpin clearly tries to minimize the exposure of the hydrophobic nucleobase surfaces to the aqueous solution.

To examine potential structural changes of D5 in a less polar medium, i.e. at a lower permittivity, we performed chemical shift mapping experiments by recording [^{1}H,^{1}H]-NOESY spectra in different D_2O mixtures containing either 5%, 10%, 15%, or 20% 1,4-dioxane-*d8*. Evaluation of this titration shows that all non-exchangeable protons are affected by the addition of 1,4-dioxane-*d8* (Fig. 2), most displaying a downfield shift. This result already clearly shows the

effect of solvent polarity as well as a possible contribution of ethylene solvation (by the ethylene units of 1,4-dioxane) on the entire structure of D5. The change in chemical shift of protons located in helical regions are thereby in the same order: By addition of 20% (*v/v*) of the organic solvent one observes for these protons a downfield shift of 0.04 to 0.07 ppm for the sugar protons and approximately 0.08 to 0.12 ppm for the aromatic ones (Fig. 2). This deshielding effect may be attributed to the lower polarity of the solvent mixture.

<<Fig. 2 close to here>>

Interestingly, regions that are implicated in tertiary contact formation and/or catalysis stick out with regard to the change in chemical shift. H1' and H8 of G3 are stronger affected by the addition of 1,4-dioxane-*d8* than the neighbouring nucleotides (Fig. 2). This nucleotide is highly conserved, forming a wobble pair with U32, and part of the so-called AGC or catalytic triad. Its minor groove site is involved in tertiary contacts whereas the major groove is implicated to harbour the catalytic site [68]. Our NOESY data under these conditions does not support a higher dynamics or even flipping out of G3, as it has been suggested previously [69], but shows an intact GU wobble pair. The stronger change in chemical shift may thus probably be attributed to the higher solvent exposure of the non-Watson-Crick base pair.

Further regions exhibiting larger chemical shift changes are the tetraloop and in particular the bulge region (U9, A24, C25 and G26): In the case of the tetraloop and its closing GU wobble pair, this is to be expected, as these nucleotides are the most solvent exposed ones of the hairpin, and are thus likely to interact with the organic solvent molecules. With regard to the bulge, the aromatic and aliphatic protons of nucleotides 24 to 26 (highlighted by an asterisk in Fig. 2) experience a distinctly stronger downfield shift of up to 0.22 ppm indicating a more pronounced exposure to the low-polarity solvent. The opposite lying A9H1' (marked by a circle in Fig. 2) is the only proton displaying an *upfield* shift under these conditions. Indeed, this might indicate some hydrophobic "ethylene" solvation [44,54] of the sugar moiety of U9. These striking deviations from the behaviour of the remaining protons already indicate structural changes in the flexible bulge

region due to the reduced dielectric environment being possibly connected with changes in solvation.

In order to get a more detailed insight into the structural changes induced by a decreased solvent polarity we carried out a detailed analysis of the [^{1}H,^{1}H]-NOESY spectra in the absence and presence of 1,4-dioxane. The addition of the organic and rather apolar 1,4-dioxane-*d8* not only causes chemical shift changes as described above but also differences in the intensity of inter- and intraresidual NOE crosspeaks within the bulge (Fig. 3). These changes already indicate a structural change within this important region of D5.

<<Fig. 3 close to here>>

As mentioned above, the NOESY spectra of D5 in D_2O show a distinct crosspeak between A24 H2 and the H1' proton of the neighbouring C25 (see also Fig. 1b) as well as between A24 and G10H1', placing the nucleobases within the helical framework and indicating a pronounced stacking among them. The A24H2/C25H1' crosspeak almost disappears in water containing 20% (*v/v*) 1,4-dioxane, while the intensities of the interstrand and most other crosspeaks remain virtually unchanged. This shows that the strong stacking between the neighbouring A and C nucleobases is weakened or even abrogated. It is well feasible that the decreased permittivity of the surrounding solvent "pulls out" the hydrophobic nucleobase plane of one or two of these unpaired nucleobases within the bulge. This would enable not only a tighter and more compact structure of the bulge region but also allow the flipped-out nucleobase(s) the formation of tertiary interactions by base stacking with nucleotides of other ribozyme domains. Indeed, D5 is embedded within the innermost core of the ribozyme architecture undergoing numerous tertiary contacts, i.e. the λ-λ', κ-κ', μ-μ', ψ-ψ', and ζ-ζ' interactions with D1 and D3 [2]. The conserved bulge thereby plays a crucial role and it is well feasible that the tertiary interactions are formed by a concerted action of the formation of hydrogen bonds and reduced permittivity upon assembly of the catalytic core.

3.2. A decreased solvent polarity increases the Mg^{2+} affinity to D5

It is well known that the stability of metal ion complexes formed with the building blocks of nucleic acids increases with a decreasing solvent polarity [52-59] (see also Section 1). However, in contrast to isolated nucleosides and nucleotides the metal ion binding properties of D5 are obviously also influenced by the secondary and tertiary structure of the RNA. Metal ion binding to such a large RNA can be characterized in detail by monitoring the change in chemical shifts of non-exchangeable protons in [^{1}H,^{1}H]-NOESY spectra [33,34]. However, the presence of 1,4-dioxane-*d8* and subsequent addition of Mg^{2+} lead to a severe line broadening of the resonances making a comprehensive evaluation difficult if not impossible. We therefore concentrated on recording [^{1}H]-NMR spectra in H_2O/1,4-dioxane-*d8* (9:1, *v/v*) and evaluated the chemical shift changes of the imino protons within D5 (Fig. 4). However, due to solvent exchange, the protons in the bulge region could under these conditions not be monitored and hence no information is gained on this part of D5. From the stack plot shown in Fig. 4 it is obvious that the chemical shifts of most of the

<<Fig. 4 close to here>>

imino protons in water containing 10% (*v/v*) 1,4-dioxane-*d8* are hardly affected by addition of up to 8 mM $MgCl_2$. Small downfield changes can be seen at G10H1 and G12H1, both located within the tandem GC region of helix 2, which is a known Mg^{2+} binding site [31].

Interestingly, the H3 of uracil-32 experiences an upfield shift being a clear indication for Mg^{2+} binding at the catalytic triad. Along the same line, G3H1 being part of this AGC triad and forming a wobble pair with U32 is also slightly upfield shifted. The major groove of such base pairs offers several perfect ligating atoms for metal ions. Mg^{2+} coordination to this crucial part of D5 could not be detected in pure water until now, and only paramagnetic line broadening studies with Mn^{2+} [31] as well as thio-rescue experiments with Cd^{2+} [70] revealed metal ion binding in this region. Hence, the reduction of solvent polarity seems to be a good way to detect Mg^{2+} binding to RNA, that is very low or even too weak to be seen in pure water, e.g. because only part of the binding pocket is present when examining single domains as is the case here with D5 in absence of

D1.

In addition, the imino protons of U14 and G19, both flanking the tetraloop, as well as of G15 show a change in chemical shift, the latter one being most pronounced of all (Fig.4). Indeed, the tetraloop is a known metal ion binding site not only within D5 [6,31] but also in general [33,34,71-73], due to its accessibility and the high content of purine nucleobases and close neighbourhood of phosphate groups.

In order to quantify the effect of reduced solvent polarity on the binding affinity of Mg^{2+} to D5, we used this titration data to estimate the stability constant of the complex formed between the tetraloop of D5 and Mg^{2+} in 10% (*v/v*) 1,4-dioxane-*d8* in water and to compare these results with those obtained in pure water. In Fig. 5 the chemical shift change of G15H1 in dependence of the Mg^{2+} concentration is shown together with the fit of the experimental data applying a 1:1 binding model. The calculated association constant log K_A of 2.44 ± 0.10 (M^{-1}) in the presence of dioxane is

<<Fig. 5 close to here>>

somewhat higher than the one measured in pure water (log K_A = 2.28 ± 0.12 M^{-1}). These constants together with the ones of the other protons that could be evaluated are summarized in Table 1. We are aware that in some cases the errors are quite high and that these constants are only a rough estimate for the following reasons: Firstly, changes in chemical shifts of imino protons may not only be caused directly by the electronic effects of metal ion coordination to the nucleobase, but also by small structural changes and an enhanced (or reduced) accessibility of solvent molecules [31]. Secondly, in order to obtain an intrinsic affinity constant for a given site, one has to take all other binding sites within the given RNA into account [34]. Nevertheless, the here measured Mg^{2+} affinity to the GAAA tetraloop in water is in the same order as determined previously for a corresponding binding site [34], illustrating the validity of our data.

The addition of 10% 1,4-dioxane-*d8* leads to an increase in complex stability by a factor of about 1.5 (see also Table 1). This amount of stabilization can now be compared with the stability increase of complexes formed by D-ribose 5-monophosphate ($RibMP^{2-}$) or related ligands and Cu^{2+}

or Zn^{2+}. The stability of such complexes increases by factors of about 1.75 (Cu^{2+}/RibMP^{2-}), 1.6 (Cu^{2+}/acetate, Ac^-) and 1.45 (Zn^{2+}/Ac^-) [52] for the same change in solvent. The good agreement between these experiments involving different metal ions and ligands and our data further corroborates our conclusions. In addition, it indicates thatmetal ion-phosphate binding is involved in the in the Mg^{2+}/tetraloop complex aside from possible N-coordination of purine residues.

Finally, one should keep in mind that the permittivity in water containing 10% (*v/v*) 1,4-dioxane-*d8* is still high, i.e. $\varepsilon \approx 70$ (25 °C) [60,61], and is thus reduced only by approximately 11% in comparison to water. Nevertheless, this relatively small difference in the dielectric constant of the medium has unequivocal effects on the complex stability between metal ions and RNA, as is illustrated by the enhanced binding of Mg^{2+} to the GAAA tetraloop of D5 in the presence of 1,4-dioxane-*d8*.

4. Conclusions and Outlook

Ribozymes and other nucleic acids are commonly studied in pure water or aqueous buffer solutions. However, as we have demonstrated in the present work, their investigation in less polar solvents is highly desirable in order to simulate the more hydrophobic environment expected in a cell. From our studies it follows that the polarity of the medium has a significant influence both on the structure of the ribozyme as well as on its metal ion-binding properties.

In the NMR solution structure of D5 clearly the bulge region deserves special interest, since it is influenced by the addition of 1,4-dioxane-*d8* remarkably stronger than the other parts of this domain. Previous results on the structure of D5 [31] showed this bulge region to be the most flexible and solvent-accessible part within this hairpin. Taken together, this means that the bulge nucleotides with their hydrophobic nucleobase surfaces can *a priori* interact best with the organic solvent molecules leading to a structural rearrangement by flipping out. Similar hydrophobic interactions, e.g. stacking or ribose-ribose contacts, are important factors for tertiary networks within complicated RNA structures. It follows that the application of 1,4-dioxane or similar

solvents can simulate such tertiary interactions, and thus either promote folding by pre-arranging local structures, or even allow to study alternate conformations of local structural motifs within RNA.

As illustrated in this work, solvent permittivity affects the metal ion-binding properties of nucleic acids. A decrease in the dielectric constant of the medium by approximately 10% causes an increase in complex stability of about 50%. This amount of stability enhancement is irrespective of the location of the metal ion binding site, be it in a helical region, e.g. at the catalytic triad, or in a loop like the GAAA tetraloop. It thus seems reasonable to assume that the intrinsic affinity of a metal ion to a specific binding pocket within a RNA can be considerably higher than the one measured under *in vitro* conditions in pure water and/or the absence of other domains. Considering the higher complex stability under conditions of a reduced solvent polarity, one can also imagine further applications of such conditions: For example, as illustrated above for Mg^{2+} binding to U32, that had not been detected before, weak interactions are considerably enhanced and become thus accessible for investigations.

To summarize, it is evident that the permittivity of the solvent is a crucial factor in bioinorganic studies, which should not be neglected including in studies on ribozymes and related nucleic acids.

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Table 1. Stability constants of Mg^{2+} binding to D5, as determined from the change in chemical shift of some imino protons and subsequent fit with a 1:1 binding model (see also text) (I = 0.1 M, 278 K). Given are the values in pure D_2O as well as in a 9:1 (*v/v*) mixture of H_2O and 1,4-dioxane-*d8* together with the enhancement factor $f_{D2O \rightarrow H2O/diox}$. The average enhancement amounts to 1.51 ± 0.32. All error limits given correspond to one standard deviation (1 σ).

	$\log K_{G3H1}$	$\log K_{U14H3}$	$\log K_{G15H1}$	$\log K_{G19H1}$	$\log K_{U32H3}$
99.99% D_2O	2.07 ± 0.08	1.98 ± 0.15	2.28 ± 0.12	2.18 ± 0.09	1.88 ± 0.16
D_2O:dioxane (9:1)	2.26 ± 0.38	2.08 ± 0.17	2.44 ± 0.10	2.39 ± 0.20	2.13 ± 0.04
$f_{D2O \rightarrow H2O/diox}$	1.56 ± 1.39	1.24 ± 0.65	1.45 ± 0.54	1.62 ± 0.81	1.80 ± 0.69

Legends to the Figures

Fig. 1. Structure of D5 from the group II intron Sc.ai5γ from *Saccharomyces cerevisiae*. a) Wild-type secondary structure with an additional GC base pair at the helix end for stabilization, as used in the present study. b) Close-up view of the bulge region. The NOEs involving A24H2 are affected most by the presence of 1,4-dioxane-*d8* and are indicated by arrows. This panel has been prepared using the coordinates from the PDB ID 1R2P [31] and MOLMOL [74].

Fig. 2. Chemical shift mapping of the H6/H8 and H1' protons within D5. Shown are the changes in chemical shift $\Delta\delta = \delta_{D2O} - \delta_{H2O/diox}$ of these resonances when changing the solvent from D_2O to D_2O containing 20% (*v/v*) 1,4-dioxane-*d8*. The bulge nucleotides A24, C25, and G26 (❍) as well as the opposite U9 are indicated (*).

Fig. 3. [^{1}H,^{1}H]-NOESY spectra (600 MHz, 298 K, 100 mM KCl) of D5 in water (a) and in water containing 20% (*v/v*) 1,4-dioxane-*d8* (b). The resonance lines of G10H1', A24H1, A24H1', C25H1', and C25H6 are indicated by dotted lines, and their crosspeaks by circles. The intensities of the crosspeaks between A24 and C25 are clearly reduced upon addition of dioxane.

Fig. 4. Stack plot of the imino region of D5 in a 9:1 mixture (*v/v*) of H_2O and 1,4-dioxane-*d8* upon increasing amounts of $MgCl_2$.

Fig. 5. Fit of the chemical shift changes of G15H1 upon Mg^{2+} binding with a 1:1 binding model in H_2O containing 10% (*v/v*) dioxane (circles, solid line) and water (squares, dashed line). The resulting stability constants are $K_A = 274 \pm 66\ M^{-1}$ (10% dioxane) and $K_A = 189 \pm 53\ M^{-1}$ (H_2O), respectively (I = 0.1 M, 278 K).

Synopsis

The dielectric constant within a cell, and even more within proteins and nucleic acids, is considerably lower than in bulk water. Here, we show that a decrease in solvent permittivity influences the structure of a RNA hairpin and concomitantly increases the affinity of Mg^{2+} to this RNA by a factor of about 1.5.

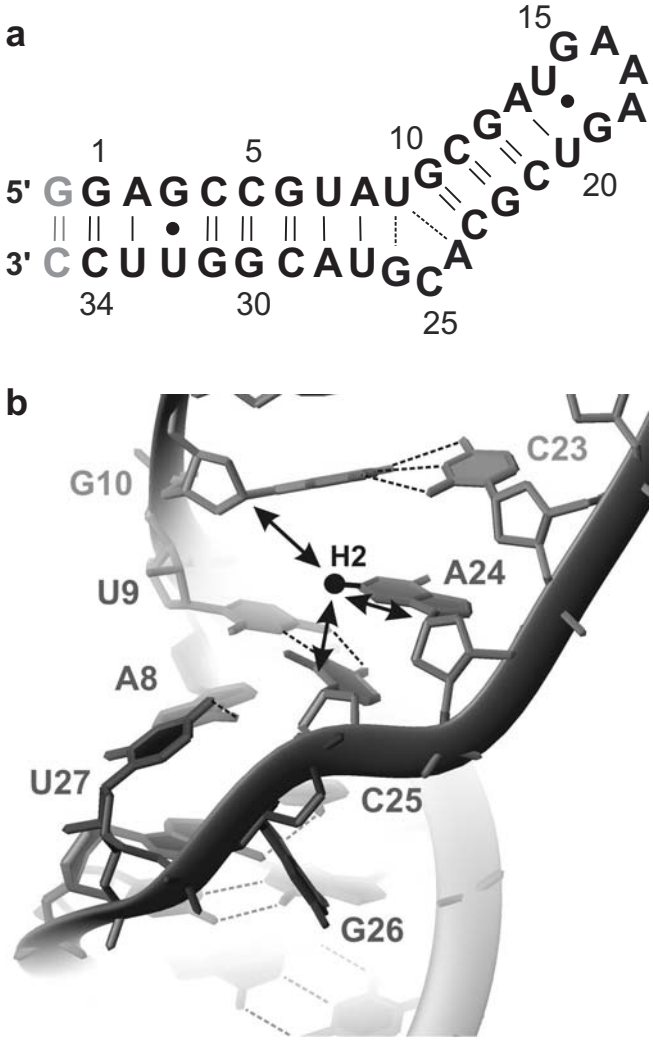


Figure 1

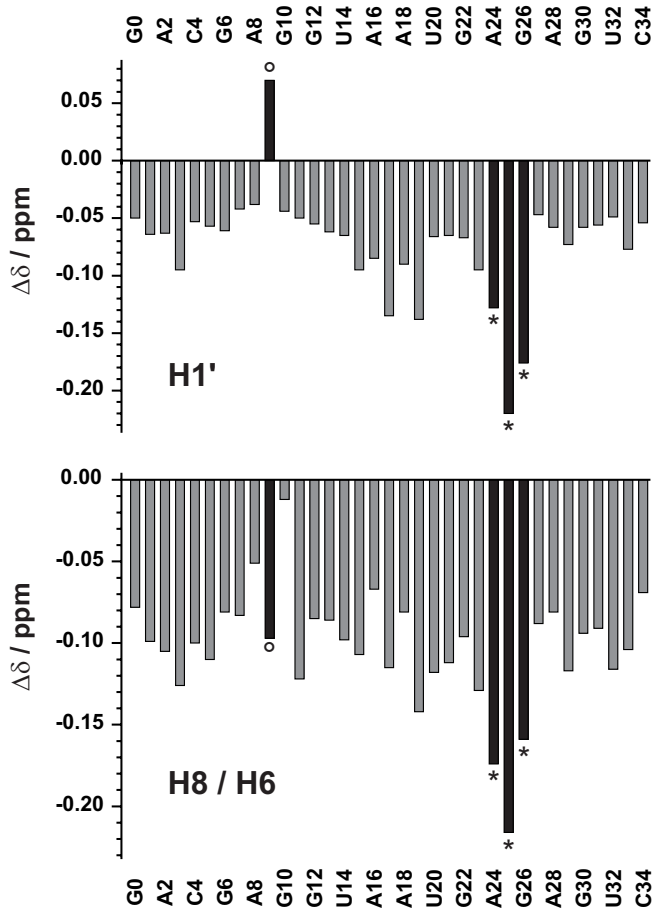

G0
A2
C4
G6
A8
G10
G12
U14
A16
A18
U20
G22
A24
G26
A28
G30
U32
C34
0.05
0.00
-0.05
-0.10
-0.15
-0.20
Δδ / ppm
H1'
H8 / H6

Figure 2

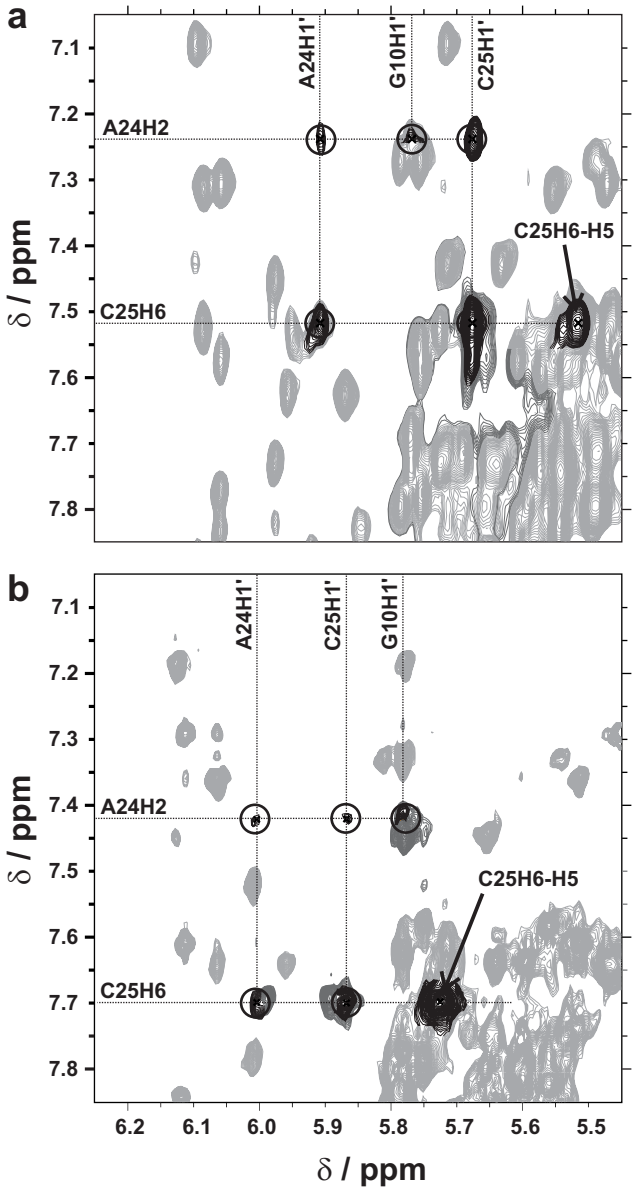

a
b
A24H1'
G10H1'
C25H1'
A24H2
C25H6
C25H6-H5
δ / ppm
7.1
7.2
7.3
7.4
7.5
7.6
7.7
7.8
6.2
6.1
6.0
5.9
5.8
5.7
5.6
5.5

Figure 3

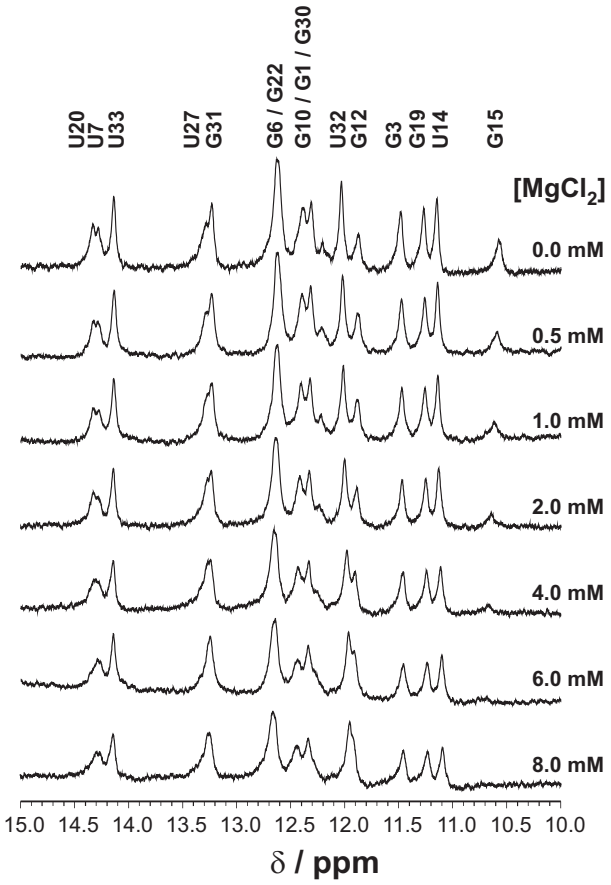

U20
U7
U33
U27
G31
G6 / G22
G10 / G1 / G30
U32
G12
G3
G19
U14
G15
[MgCl2]
0.0 mM
0.5 mM
1.0 mM
2.0 mM
4.0 mM
6.0 mM
8.0 mM
15.0 14.5 14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0
δ / ppm

Figure 4

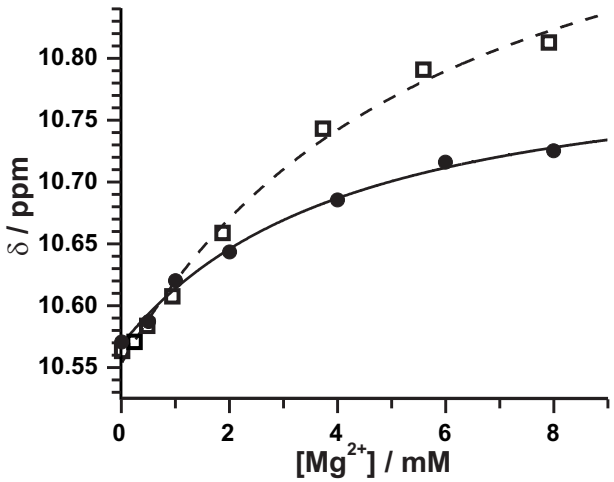
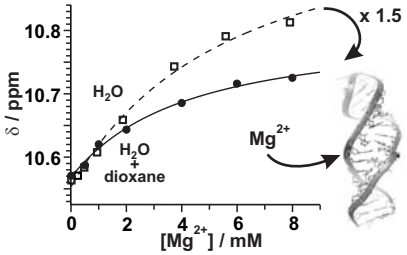


Figure 5



Graphical Abstract